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OBSERVATIONS AND EXPERIMENTS BEARING ON
“ISLE OF WIGHT” DISEASE IN HIVE BEES.

BY

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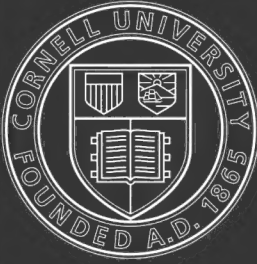
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IV—Observations and Experiments bearing on “Isle of Wight” Disease in Hive Bees.¹ By John Anderson, M.A., B.Sc., Lecturer in Bee-keeping, North of Scotland College of Agriculture, and John Rennie, D.Sc., F.R.S.E., Lecturer in Parasitology, University of Aberdeen.

(With Plate.)

(Read 24th January 1916. MS. received 11th February 1916.)

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I.—INTRODUCTION.

THESE observations were commenced in 1909 by one of us (J.A.), whose journal contains a detailed record of the history of each stock of bees in the Island of Lewis. In the autumn of 1913 J.R. paid two visits to the Nicolson Apiary at Stornoway, and subsequently arrangements were made, through Professor J. Arthur Thomson, for continuing the research in association with the Natural History Department of Aberdeen University.

The work here reported on was carried out with the aid of grants from the Development Fund and the University of Aberdeen through the Joint Committee on Research in Animal Nutrition of the University of Aberdeen and North of Scotland College of Agriculture. In the work we have been assisted by Mr John Innes, B.Sc., M.B., who has carried out most of the examinations of bees for the presence of *Nosema*, and

¹ This constitutes a preliminary report, indicating the trend of the results so far accomplished. Although the number of experiments is not large, stress is laid upon the fact that they are the only ones on record in this country which have been made upon full stocks of bees living under natural conditions. Further experiments are in progress, the results of which will be recorded in a subsequent report.

after his retiral on taking up military service, by Miss Beatrice Simpson, M.A., who continued this work. Mr Robert Ewen, M.A., Nicolson Institute, Stornoway, has acted as local supervisor of the hives in Lewis since May 1915. We have also had the valued co-operation, in connection with the Deeside epizootic, of Mr A. H. E. Wood, of Glassel House, and of Miss Nancy Robinson, both of whom are bee experts of the British Bee-Keepers' Association. The latter has provided a detailed record of the Deeside outbreak, which has proved of value as a basis for scientific study.

We are indebted also to various local observers in Lewis and elsewhere who have taken charge of experimental hives, forwarded diseased bees, and otherwise supplied helpful information regarding the disease.

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II.—THE COURSE OF THE DISEASE, AS OBSERVED IN THREE DISTINCT LOCALITIES.

(a) LEWIS OBSERVATIONS.

Prior to the 20th May 1909, all hive bees in Lewis had become extinct, but on that date one stock was brought to Stornoway and placed in the grounds of the Nicolson Institute. These were black bees obtained from Wormit, Fife, and their previous history is unknown. In autumn of the same year a second stock of black bees was imported from Duirinish, and placed at Bayhead, Stornoway. Since 1909, other bees have been introduced at various dates, as stocks or swarms, and some queens have also been imported. At the present time there are bees in Stornoway, Lurebost, Sandwick, Bayble, Tong, Tolsta, Barvas, Shawbost, Carloway, Breasclete, Achmore, Marybank, Laxdale. The races include Blacks, Italians (Ligurians), Cyprians, Carniolans, and certain hybrids.

First Appearance of Isle of Wight Disease.—On the 20th September 1910, two lots of driven bees arrived in Stornoway from Sussex. They had come from cottagers' skeps, but had travelled to Lewis in swarm boxes on standard combs containing some honey. These standard combs probably came from the apiary of the bee-master who "drove" the bees, and it is possible that the disease was derived from these combs. One box was sent unopened to Bayble, 5½ miles from Stornoway, and the other lot was retained in Stornoway. The bees in both cases were fed on syrup, settled down quietly, bred freely, and gathered pollen up to 4th November 1910.

On 31st January 1911, bees were observed crawling in front of this stock,

apiary number, A9. Next day a letter was received from Bayble stating that the other stock of driven bees had begun to crawl on the same day. The symptoms in both cases were those of Isle of Wight disease; bees emerged on the alighting board and did not fly, some eventually fell off the board on to the ground where they collected in little clusters, finally dying of cold and hunger. Bees of this stock continued to die in this way at Stornoway till 2nd March. On this date the survivors were killed and the hive and ground disinfected. Caustic soda was spread upon the soil, which was then turned over, and all the combs and quilts were burned. The Bayble stock was also killed off and the hive disinfected.

Second Attack. Two Stocks affected, B14, C13.—On the 6th October 1911, two other stocks in Stornoway Apiary began to crawl. On this occasion, samples of living bees were sent for diagnosis to Dr Graham-Smith, Cambridge, who reported “young stages of *Nosema apis* in both stocks.” Spores were apparently not observed, and it was not stated whether the examples seen included intracellular forms. One of these stocks (B14) consisted of American Golden Bees, and in their case crawling ceased after a few days; on 14th October they were busily engaged once more carrying in pollen. They wintered in excellent condition, built up rapidly in spring, and never showed any further sign of Isle of Wight disease. They were not again tested for *Nosema*. Unfortunately the queen died of old age, 15th June 1912, and the strain became extinct. The noteworthy feature in the case of this stock is that the symptoms, although associated with the presence of *Nosema*, disappeared in a few days.

The other stock (C13) which consisted of black bees, became rapidly worse, and, on 12th October 1911, it was destroyed. These two stocks stood side by side.



Arrangement of Stocks, October 1911.

Third Attack. One Stock, No. D1.—Crawling bees were first observed in front of this stock, the position of which is indicated above, on 20th January 1912. The bees were a black stock, numerous, and having abundance of natural stores. It had wintered in excellent condition. Samples were again sent to Cambridge, and Dr Graham-Smith reported the presence of all stages of *Nosema*.

This stock was allowed to die out naturally, but the hive had been removed to another site, and the original stance disinfected. A small remnant was found stiff and motionless on one comb, on 29th February 1912. Thorough disinfection of the site was effected by spraying crude carbolic acid, of such strength that the worms came up and died, and the grass also was killed. All inside fittings of the hive were burned, paraffin was also poured on the hive site, and burned off.

Fourth Attack. Stock D4.—This stock, a strong one, crowded with bees and brood, along with two others, came from Exeter on 21st May 1912, where as yet the disease was unknown. It was placed on the stance of D1, referred to above. Isle of Wight disease symptoms appeared on 28th May, exactly one week after the arrival of the stock upon the island, and large numbers of crawling bees were observed in front of this hive on every fine day. On 8th June, all the stores were exhausted and all the brood was dead. Syrup was supplied, and the queen laid more eggs. On the 13th June there were still numerous bees, eggs, a few grubs, and food. At this date samples were sent to Cambridge, and Dr Graham-Smith's report upon them is as follows:—

"The bees you sent all arrived dead. No spores were found by microscopic examination, and it was impossible to say for certain whether young stages of *Nosema* were present or not. I tried to infect some healthy bees, but this experiment has turned out negative. 2/7/12."

On the 17th bees were lying about in large numbers on the alighting board and on the grass around, and dying off rapidly.

On the 11th July, the remaining bees, along with queen, were sent to Dr Graham-Smith, who kept them for some time. His report regarding them is:—

"The bees you sent have been doing moderately well, and nothing very special has yet been found in those which have died."

This must mean that *Nosema* was not found, yet the behaviour of the bees, and the high mortality clearly indicated Isle of Wight disease.

In the case of this stock no disinfecting measures were adopted, nor in any subsequent instance in the Nicolson Apiary, Stornoway.

Fifth Attack. One Stock (K.M.).—This stock arrived in Stornoway from the South of England on 31st May 1912. Its ten combs were crowded on 11th June and a super was added. The super was quite full of bees on 22nd June, and some sections were ready for sealing on 13th July. Later a

second super was required, and some excellent sections were removed. Crawling began on 10th September, and was very typical when observed on the following day. On 12th October, all the bees were dead except the queen and a handful of workers. This stock was situated about 200 yards distant from the Nicolson Apiary.

Sixth Attack. Stock E14.—This stock consisted of Swiss pedigreed bees (blacks), and was imported from Exeter on 4th June 1913. When a super was added on 9th June the hive was crowded with bees and brood. When examined nine days later the super was crowded with bees, the foundation had been drawn out, and some honey stored in the cells. The stock had been placed in a garden which had not previously been used for bee-keeping, a short distance from the Nicolson Apiary.

On the 19th, bees were observed crawling in front of the hive, exactly a fortnight after their arrival in Lewis. Numerous bees were examined on the spot, their intestinal contents being searched microscopically. *Nosema* spores were not found. Sick bees were sent to Cambridge, and regarding these Dr Annie Porter reported: "A very few spores in one bee, young stages in some of the others, the rest negative." Notwithstanding the apparent paucity of *Nosema*-infected bees, the mortality amongst members of this stock continued, and at this period was exceedingly heavy. On 7th July, the Swiss queen was replaced by a Cyprian imported from Nicosia. The Swiss queen was caged with some Cyprian bees, and died of injuries received from them. She was sent to Dr Annie Porter, Cambridge, for examination, but no report was obtained regarding her condition. By this time, not many blacks were left alive; by September the stock was weak in numbers, but apparently healthy, and very few blacks were present. On 16th September, one of these was found to contain spores in abundance, but no spores were found in the young Cyprians. A few of these were observed to "crawl" on fine days, but the stock proved too weak to winter, and was allowed to die out.

Seventh Attack. Two Stocks affected, Gp. and Gs.—A stock of black English bees had been brought to Stornoway from the mainland of Ross (Duirinish) in the autumn of 1909. It prospered normally during 1910, 1911, and 1912, producing several swarms, and giving surplus honey. In 1913 it swarmed on 25th July. "Crawling" began in the swarm three days after it was hived, and was well marked and typical when observed on 16th August. On this date a few crawlers were observed in front of the parent stock. On 6th September, when the apiary was again visited no crawlers

were found in front of the swarm, but a large number was seen in front of the parent stock. Both hives were examined comb by comb on this occasion, and found to have queens and brood in all stages. The swarm was small in number, but lively, and apparently quite healthy. The parent stock was more numerous, but a considerable number of bees showed dislocation of the wings, an indication which, however, has little importance as a symptom of the disease. On the 12th September, sample bees were taken from the parent stock. These were picked off the combs, bees with dislocated wings being specially selected. Owing to unfavourable weather conditions there were no bees out of doors at this date. Microscopic examination showed no recognisable traces of *Nosema*.

The hives in which the bees were housed had no previous history of Isle of Wight disease. The bees were too few to keep warm in the hives during winter, and, on this account alone, survival was not possible.

Eighth Attack. One Stock, F14.—Without any disinfecting measures, Hive No. 14, which housed the previously mentioned infected stock (E14), was used for a lot of Welsh driven bees procured in the late autumn of 1913. The infected combs were also retained with contained honey. In early spring, 1914, heaps of dead were found on the floor; later, on fine days many "crawlers" were observed. At the beginning of April, the bees covered only a single comb, but they were carrying pollen freely, and there was a patch of brood. Crawling had now ceased, the stock built up during the summer of 1914, wintered on natural stores, and was quite strong in the spring of 1915. On 17th June 1915, J. R. examined six live bees, active after journey (posted 14th), all parts normal, no *Nosema* observed. Other samples of bees which were sent to Aberdeen for examination arrived dead, and were not satisfactory for Isle of Wight disease diagnosis. On the other hand, it can be stated positively that *Nosema* spores were not present in the bees examined. Bees were examined individually, and also pulped in groups. The colons contained masses of pollen, but *Nosema* was not found. A stained smear was also made of one bee, and this gave a negative result.

In August 1915, the stock was found weak and queenless. In October it was quite extinct, and the local observer (Mr Gibson) reported that crawling had been very marked.

In October the hive was restocked with a new lot of driven bees from Wales, the old combs and stores being retained. Efforts were made to put this stock in good condition for wintering, but the weather was rather cold while feeding was going on.

This is now the third stock housed in the same hive in which two previous

stocks had died of Isle of Wight disease. This new stock and hive is named G14.

No further cases of Isle of Wight disease have arisen in the Nicolson Apiary, although, as the records below show, *Nosema* could be obtained from bees there at almost any time.

Outbreaks of Isle of Wight Disease in Lewis.

Date of Introduction of Stock	Date of Outbreak	Stock	<i>Nosema</i> x, o, or y.s.	How terminated	If Site, etc., disinfected
29/9/10 from Sussex	31/1/11	Blacks A9 and Bayble	—	Destroyed 2/3/11	Yes
	6/10/11	American Goldens B14	y.s.	Recovered	—
	6/10/11	Blacks C13	y.s.	Destroyed 12/10/11	Yes
	20/1/12	Blacks D1	x	Allowed to die out 29/2/12	Yes
21/5/12 from Exeter	28/5/12	Blacks D4	?	Remainder sent to G-S., Cambridge	No
31/5/12 from S. Eng.	10/9/12	Blacks KM	—	Died out 12/10/12	Yes
4/6/13 from Exeter	18/6/13	Swiss pedigreed E14	Very few spores in 1; numerous in Sept.	Died out before Winter	No
Autumn 1909 from Duirinish	28/7/13	Blacks Gp	o	Died out Winter 1913-14	No
Autumn 1913 from Wales	Early Spring 1914	Welsh F14	o	Recovery in 1914 Relapse in 1915	No
11/7/14 from Exeter	12/7/14	Black (at Bayble)	o	Blacks died out in Autumn; remnant of Yellows survived Winter	—

The number of stocks in the apiary varied from 4 to 14 during this period.

x—spores present. o—spores absent. y.s.—young stages.

Summary of Characteristics of Isle of Wight Disease as seen in Lewis Outbreaks.

(1) *In relation to area beyond the Island.*

- (a) Simultaneous outbreak of two cases $5\frac{1}{2}$ miles apart of stocks introduced from same source (Sussex), 4 months previously. A9 and Bayble stock.

- (b) D4. Disease appeared 1 week after stock arrived from Exeter.
 E14. „ 2 weeks „ „ „
 KM. „ 3½ months „ „ S. England.
 F14. „ 6 months after introduction from Wales.

See also under (2).

(2) *In relation to other affected stocks in Lewis.*

- B14. } More than 7 months after the termination of the previous
 C13. } outbreak.
 D1. Three months after B14 and C13.
 F14. Six months after introduction, upon which it had been placed
 in hive in which a stock, E14, had just died out from
 Isle of Wight disease.

(3) *Duration, where not interfered with.*

- B14. A few days, and recovered.
 D1. 40 days, and died out.
 KM. 32 days, and died out.
 E14. June to beginning of winter, and died out.
 Gp. July to winter, died out.
 F14. Few weeks—recovery—recurrence in the following year,
 lasting August to September 1915.

(4) *Relation to Nosema apis.*

1. Spores found in only two cases coincident with Isle of Wight disease symptoms.
2. Young stages were found in two other affected stocks, one of which completely recovered.
3. Healthy stocks known to have *Nosema* all the time in which no outbreak of Isle of Wight disease occurred.
4. A particular case,

{ Placed on a new site when introduced.
 Strong stock.
 Mortality heavy.
 Extreme paucity of *Nosema* in bees while this mortality in progress.
Nosema fluctuations at different stages.

(5) *Effect on Apiary as a whole.* No more than two stocks ever displayed symptoms at one time or in succession. During this period the apiary contained various races and strains of bees.

(b) EXAMPLE OF NATURALLY OCCURRING OUTBREAK OF ISLE OF WIGHT DISEASE, SHOWING SPONTANEOUS RECOVERY.

CRAIBSTONE APIARY.

On 4th June 1915, three strong stocks of bees imported from Devonshire were placed in this newly constituted apiary and housed in perfectly fresh hives. During the fine weather of June they were very prosperous, each stock swarming once and one of them twice. The swarms were hived separately. Three races were represented, viz. pure Italian, pure Carniolan (queen imported from Austria in 1914), and ordinary black bees.

Symptoms of Isle of Wight disease made their appearance on 30th June, and continued, with fluctuations, till near the end of August. All the stocks and swarms were affected more or less, but the heaviest mortality occurred in the blacks (D4). Three of the seven stocks died out owing to their failure to mate the virgin queens. In all three cases drone breeders were developed. On 22nd September four stocks survived and no symptoms of Isle of Wight disease were apparent, either in the behaviour of the bees, which was perfectly normal, or in the condition of their internal organs. At no stage of the disease were spores of *Nosema* found, although repeated examinations of the contents of the alimentary canal were made.

While the disease was at its height the number of affected bees was very great. Their behaviour was of the usual character, and the walk leading from the hives was littered with crawling bees to a distance of at least 25 yards. Heaps lay on the flower-beds, and a low box-hedge was full of bees incapable of flight.

The mortality was, in consequence, very considerable, and the weather conditions were exceedingly unfavourable to bee life. The recovery is therefore all the more notable.

On 22nd September one of the stocks (D4) was decidedly weak. It was headed by a young queen, had brood on two combs and plenty of store for the number of bees. This is the stock which had the heaviest mortality during the crawling period. Two other stocks (A1 and C3) were in excellent condition for wintering, headed by young queens, populous as to bees, and with plenty of stores. The fourth stock (B2) is the only one now that is not headed by a queen of this year. Possibly on this account, and also on account of heavy losses during the crawling period, this stock is also rather short of bees. The queens in D4 and B2 are black, C3 has a young Carniolan queen. A1, originally pure Italian, is now filled with dark bees, the offspring of a young queen.

On 23rd September, a small lot (3 lbs.) of driven bees was united to D4, and a new stock, E5, consisting of a small lot of Welsh driven bees with their own queen, was placed between C3 and B2. On 10th October, an Italian queen, supplied by Penna, was caged over D4, the original queen having been removed, and this Italian queen was duly accepted. On the same date it was observed that B2 (the weakest stock in the apiary) had been entirely robbed out. D4 and E5 were fed with sugar syrup and duly wintered down.

On 28th December, the four Craibstone stocks were examined. Except at A1 there were scarcely any dead bees to be seen, either on the ground, on the alighting boards, or on the floors of the hives. Even at A1 the number of dead bees was not remarkable.

D4 was opened, and the bees were found active and in good condition generally.

During the mild days in January 1916 the bees were moving freely and even carrying pollen. The behaviour of C3, E5 and D4 was normal, but A1 showed very definite "crawling" symptoms and a high death-rate.

(c) ISLE OF WIGHT DISEASE ON DEESIDE.

1.—HISTORY OF OUTBREAK.

By NANCY M. ROBINSON, First-Class Expert, B.B.K.A.

So far as can be ascertained, Isle of Wight disease was not recognised anywhere on Deeside until 1913, when it appears to have broken out in several distinct districts.

(1) On *Lower Deeside*, in *Culter* and the surrounding districts, the disease was at its worst in 1914.

A.—A's apiary was the first affected. He had bought bees from the South of England in 1912. These stocks did well early in 1913, but they had died out by 1914 after showing all the usual symptoms of Isle of Wight disease.

In 1913 these stocks had been taken to the heather, east of the Hill of Fare, in the Echt district, and several apiaries there, including two large ones, were badly affected by the same disease in 1914 and 1915.

During 1913 and 1914 all bee-keepers within a radius of two miles or more from A's apiary found their bees affected or dying off rapidly, and by the end of 1914 there were no bees left in that area.

Some apiaries were started again but with little or no success, owing to the recurrence of the disease.

In 1915 cases of the disease appeared still farther afield and embracing a far wider area.

Drumoak.—In the parish of Drumoak, on the north side of the Dee, a case of Isle of Wight disease occurred in 1913.

B.—B had sent to England for driven bees in 1912, and in the spring of 1913 the disease first began to show in his apiary.

During this year his six stocks gradually dwindled and died out. The hives were cleaned up and disinfected.

As far as is known no further cases occurred in this parish in 1913, unless it might be at H's, which is mentioned later. But in 1914 all the apiaries in B's vicinity became affected.

C.—C lived about a quarter of a mile north of B. In June 1914, C reported that his bees were not working, although the weather was good and there was plenty of nectar to be had. The apiary, consisting of two hives and a ruskie, was found to be in the final stages of the disease, and in July there were very few bees left and less honey than in June. The owner then destroyed his bees and disinfected his hives.

D.—D was another neighbour, living about three-eighths of a mile north-east of B. He had only one stock, which was found to be very weak in June 1914. The stock was left until August, when it was so much reduced that the owner destroyed the few bees that were left and disinfected his hive.

E.—E's apiary, situated three-quarters of a mile north-east of B, was attacked in August 1914. The bees may have been suffering earlier, but no record of this exists. E had sent samples of bees to the *British Bee Journal*, which reported them to be suffering from Isle of Wight disease. Incidentally, it may be mentioned that bees from the same source sent to Aberdeen University investigators were reported as free from *Nosema*. Only two stocks appeared to be infected, but in September 1914 the remainder showed marked signs of the same trouble. In October three out of four stocks were seriously affected, and between this time and April 1915 these three stocks had died off, and the remaining one was so weak that there was merely a handful of bees on the comb. These the owner treated with candy and quinine without success, and the stock died out before the end of the month.

F.—F lived about a mile north-east of B and owned four stocks. In August 1914 there were slight indications of Isle of Wight disease. In September one stock became badly affected, and the three others all showed symptoms. The worst of the affected stocks was removed to Aberdeen for experimental purposes (60 DD. of this report). The *British Bee Journal* reported

on samples of bees sent: "It is Isle of Wight disease." Dr Rennie reported: "*Nosema* can not be found in this stock."

The remaining stocks were not destroyed; but all died out except one, before April 1915. This stock was then very weak and was destroyed, and the hives and appliances were disinfected.

G.—G kept two stocks of bees, about three-eighths of a mile east of B. In June 1914 both were very strong; but at the end of June, drones were being thrown out in great numbers, and later in the year the bees were suffering from Isle of Wight disease. Before April 1915 both stocks were dead.

H.—H's house was quite close to G. In the spring of 1914 H had an empty hive in his garden; it was filled with old combs and had been left uncared for since the loss of a stock during the previous winter. (The symptoms accompanying the loss of this stock were unmistakably those of Isle of Wight disease. *J.R.*) In June a swarm came and took possession of this empty hive and flourished remarkably. 1914 proved a good season for honey, and this stock came through the winter, 1914-1915, very well. When visited in April 1915, the bees were found working vigorously and very strong for that time of year.

A swarm came off at 9.30 A.M. on 22nd May (the earliest swarm known on Deeside that year). H succeeded in skepping it, but unfortunately it rose later and was lost.

In due time a second swarm came off, which was skepped successfully, and stock and swarm both appeared to thrive.

Later, this swarm showed distinct signs of Isle of Wight disease during two or three days, and then it seemed to recover. At the end of the honey season, the two stocks were united. For further history of the united stock, see p. 59 of this report.

J.—J, three-quarters of a mile south-east of B, kept two stocks of bees, No. 1 and No. 2. When visited in June 1914 both stocks were found very strong, but No. 1 was especially good and was working well in the supers. In July large numbers of drones were being thrown out of the hive, in spite of fine weather and a good honey flow.

In August No. 1 showed marked signs of the disease, and J then destroyed the bees. This stock had given him 126 sections.

No. 2 swarmed, and the old queen having been killed the swarm was returned to the parent hive. A young queen mated successfully, and this stock did very well in 1914, but died out during the winter 1914-1915.

It may be mentioned here that J had earlier in the season assisted some of his neighbours in destroying their infected bees and cleaning and disinfecting their hives.

In 1915 J resumed bee-keeping. He obtained a swarm and placed it in one of his disinfected hives. This stock built up steadily at first, later it began to show some symptoms of the trouble, and then partially recovered.

K.—K lived a mile south-west of B. He had only one stock, which became seriously affected with Isle of Wight disease during the summer of 1914. It was destroyed in September 1914.

In 1915 numerous outbreaks occurred farther afield from B's apiary. There were several cases about $1\frac{1}{2}$ to 2 miles north of B. Many of the stocks had died out during the winter, and not from starvation as there was plenty of honey in the combs. Those stocks which survived soon began to show the ordinary symptoms of Isle of Wight disease, and all died out. B's stocks, it may be noted, were cleaned out and disinfection effected in 1913.

On the south side of the Dee, opposite Drumoak, two cases were noted in 1915.

L.—L lived rather over 2 miles south-east of B's apiary, and owned six hives and two ruskies. In June 1914, all were strong and apparently well, but owing to the prevalence of Isle of Wight disease in the district, L was warned to watch his bees carefully. No symptoms were recognised until June 1915; all the stocks were then strong, but later they became seriously affected and eventually died out before the end of the year. L dealt a good deal in so-called "remedies."

M.—M's apiary was 2 miles south of B. In May 1915, several of his stocks were found to be affected. M was inclined to think the disease was derived from some bees from Culter, which had been brought to the heather near by, in August 1914, and which had died out while there.

(2) *Banchory-Ternan District.*

In this district the distance between the outbreaks has been greater than in the others.

N.—A case occurred at Crathes in June 1914. N's apiary lies about three miles east of B. There were three stocks, Nos. 1, 2 and 3. The following observations were made:—

13th June.—A warm, bright day. There were numerous dead bees outside the hives, and a great many unable to fly and crawling on the ground all over the garden. No clustering was seen, and no old bees observed crawling. The wings and abdomens appeared to be normal, but the bees seemed weak. Stocks all seemed strong.

15th June.—A fine day. Only a few bees were seen crawling, and very few dead bees in front of the hives. Birds were seen to pick some up, and possibly numbers may have disappeared in this way.

16th June.—A fine day. Bees appeared normal. No. 2 looks like swarming. No crawling bees seen.

21st June.—A dull, rather cold day. Bees were clustering on the ground near all three hives in small groups. No. 2 appeared to be the only normal stock, and was strong. The bees from No. 1 and No. 3, both inside and outside the hives, appeared weak and dormant and unable to sting. There were very few dislocated wings. The abdomens and general appearance of the bees, with a few exceptions, seemed normal. It is doubtful whether any bees from No. 2 were clustering, although there were clusters under that hive.

22nd June.—A fine day. The symptoms just as previous day. No. 1 and No. 3 stocks were destroyed and the hives disinfected.

24th June.—No 2 was working well and looked like swarming. The ground was limed.

26th June.—No. 2 working well.

29th June.—Swarm came off, but got away and was lost.

9th July.—Bees working well, but some few drones observed crawling.

15th August.—No. 2 working well and seems strong. Supers nearly full.

This stock died out during the winter 1914-1915. All stocks belonging to three bee-keepers, who were near neighbours of N, were lost also.

O.—O's apiary was 2 miles north of N. O had six stocks, and in July 1914 these were all found to be suffering from Isle of Wight disease. Only one stock survived till 1915, and that eventually died out.

Several cases were reported at Raemoir in 1914 which were not verified as the stocks had been destroyed before notice was received.

In 1915, there were two outbreaks, three-quarters of a mile distant from each other, at this place.

P.—P had five stocks in good hives, clean and well kept. The stocks were all strong. All appeared healthy in June 1915, but in July Isle of Wight disease was very marked. Numerous bees were crawling on the ground and clustering; others were climbing up grass and leaves; many had dislocated wings and distended abdomens. In those examined the colon was very much enlarged and clogged. There were many dead lying about.

Three of the hives contained parent stocks, while the other two held swarms. The parent stocks seemed to be more severely attacked. P destroyed all his bees at the end of July and cleaned and disinfected his hives and appliances.

Q.—Q's apiary was not so well kept, and his stocks were weak, consequently the outbreak did not appear so marked, and the disease lingered on for a long time. I have frequently noticed that when a really strong

(i.e. numerous) stock becomes affected, disease progresses much more rapidly than in the case of weaker stocks.

R.—A serious outbreak occurred in July 1915, in R's apiary, situated about a mile and a half south-east of N's apiary. R owned one hive and five ruskies, and by the end of 1915 nearly all his bees were dead.

S.—This brings us within a mile of Banchory village where a case broke out in July 1915 in S's apiary. This was on the east side of the village and within a mile of the last outbreak at R's.

S owned five hives and a ruskie. In May three stocks were very weak. Numerous dead bees lay near the hives, but this might have been the result of robbing.

In July, Isle of Wight disease was marked in a swarm in this apiary. In August all the other stocks but one began to show the same symptoms, and S destroyed them. The one left was strong and gave two crates of sections, which was good for a poor season.

At this date no cases farther west of Banchory and Raemoir have come under notice until Dinnet and Tarland are reached—15 miles away.

(3) *Dinnet Area.*

T.—In 1913, two bee-keepers at Dinnet, very near neighbours, found their bees suffering from some trouble they were unable to recognise. They consulted the North of Scotland College of Agriculture bee expert (Mr Manson), who found the bees to be suffering from Isle of Wight disease. His opinion was confirmed by the *British Bee Journal* expert, to whom samples of bees were sent. The owners were recommended to destroy their stocks in order to prevent the spread of infection. They not only did this, but burnt up bees, hives, and all appliances. One bee-keeper, T, owned fourteen stocks. Before the disease was recognised a swarm had come off and settled in the roof of a cottage close at hand. This swarm seemed to be strong and working well; and owing to the difficulty of removing the bees, it was left alone. T had brought bees from England not very long before the outbreak, and the disease was attributed locally to these imported bees.

In 1914, T started keeping bees again. He had made himself new hives and bought two stocks from a bee-keeper west of Ballater. In July 1914, he had six hives and three ruskies; all were very strong and doing well in the supers, and there was no appearance of disease.

In October 1914, the stocks seemed in splendid condition, and T was also feeding two stocks of driven bees. These had been obtained locally.

In the spring of 1915 he reported that several stocks had shown marked signs of dysentery, but there were no crawlers. T disinfected his hives after the attacks of dysentery.

In the spring of 1915 the swarm of bees already mentioned, which had settled in the roof of the cottage in 1913, showed marked symptoms of the disease. T, with the help of neighbours, promptly removed the bees, disinfected the place, and blocked up the entrance.

In June 1915, T's stocks were strong and working well.

In August 1915, one stock showed very slight signs; there were very few crawlers, and it is understood that T meant to destroy it.

U.—U's apiary is situated about half a mile due east from T.

In 1914, he owned seven hives and five ruskies.

In October 1914, there were no signs of Isle of Wight disease; but robbing was going on very vigorously. On this account some stocks were probably weak.

During the following winter, 1914-1915, five stocks died out, and all showed signs of dysentery in the early spring.

In June the remaining stocks were fairly strong, and all combs were badly marked with dysentery. When visited the weather was dull and no crawlers were seen.

The following day was bright at intervals, and a few crawlers were then observed. These were found to have the colon very much dilated and clogged.

In August 1915 only one stock remained, viz. the one which had appeared the weakest in June. It showed slight but distinct traces of the trouble, and U decided to destroy the bees and clean up and disinfect his hives. He did so in order to prevent the disease spreading among his neighbours.

No other cases in the immediate neighbourhood have been reported, although there are several apiaries near by.

V.—V's apiary is situated about five miles north-east of T. It consisted of ten hives and twenty-five ruskies.

In August 1915 all seemed very strong and well cared for, but examination showed the ground covered with bees unable to fly, with distended abdomens, dislocated wings, and all the usual appearances of Isle of Wight disease. I could not locate any special stocks as being the affected ones, for the bees were distributed all over the grass, and not in front of any particular hives. Samples of bees were sent to Aberdeen University investigators as usual, who reported that the appearances were those associated with the disease. The bees sent did not contain *Nosema*.

A week later, I found only three hives and one ruskie left. The rest had been destroyed on receipt of the above-mentioned report.

The surviving stocks were examined, but no symptoms of Isle of Wight disease were apparent. The owner, as a precaution, however, destroyed them.

X.—X, situated about one mile north of V, had originally come from Strathdon, and his bees had never flourished, but had been continually dying out. V had been down to work among them several times in 1915, and used his own gloves, smoker, and veil.

Drones had been thrown out of one of X's stocks abnormally early, and V had come in order to advise. Later, a first swarm came off this stock, and died out very shortly afterwards. A second swarm then came off, and these bees also died off.

When the stock was visited in August 1915, there was merely a handful of bees left. X then destroyed these, his last stock, and cleaned and disinfected his hives.

Y.—In 1914 X had sold an old hive to a neighbour Y, who lived about two miles south of his apiary. The stock which was placed in this hive died, and another stock beside it was reported dying out also.

No other cases within a radius of five miles are known.

2.—EXTRACTS FROM DIARY OF VISITS OF J. R. TO DEESIDE BEE-KEEPERS.

23rd August 1915. Visited Anguston Apiary.—Found twenty-one hives in small garden all affected with Isle of Wight disease. Ground littered with dead bees so that it was not possible to walk in garden without treading on them. Some had been treated with a syrup of sugar and jalap, and were reported to be improved, but this was not permanent. Sample taken; pulped. Negative result as regards *Nosema* spores.

Visited R's Bees at Drumoak.—Both stock and swarm active. Swarm had required an eke. There were a few crawling bees about, but not many. This is a case where intermittent crawling, moderate only, has occurred. Is this an aspect of the resistance of this stock where the infection has laid hold of a few, but has not overcome the majority of the stock?

D—, *Durris.*—When last seen, one stock had Isle of Wight disease; now, four or five have it. He has been spraying ground with Izal, and feeding an advertised preparation to the diseased stock, but apparently without effect. The disease has spread. Bees examined did not contain *Nosema* spores. When visited again in January 1916, all the stocks were extinct.

24th August. Visited Cullerlie, K's Apiary.—Isle of Wight disease prevalent in most stocks. No. 10 has got it. But the Italians, Nos. 25 and 18, are both going on well with no signs. Hybrids also are well, and these have swarmed lately. This swarm has settled in the top of a hive at Woodside which has got Isle of Wight disease very badly. I saw both stocks working (above and below) from the same hive. Took sample from No. 10. Result—failure to find *Nosema*.

H—, Finnercy.—Has Isle of Wight disease in most of his stocks. Took a sample from the worst, but did not find *Nosema* spores. Examined all K's sample, also H's. All had congested colons with pollen in a pasty condition. Most had chyle-stomachs, purplish; some were quite dark.

Main Features of Deeside Epizootic.

This report deals with twenty-three apiaries extending from Culter, near Aberdeen, to Dinnet, a stretch of about 30 miles along the Dee valley, and covering 3 or 4 miles on either side.

- I. The epizootic was observed first in 1913, in particular at Culter and Dinnet, and in stocks introduced from the South of England. In the Culter case one year at least elapsed between the time of arrival and the commencement of the outbreak. The disease is still prevalent in this district.
- II. Within the area there has been a very considerable amount of destruction of bees and appliances, of disinfecting generally, and also of experimenting by the bee-keepers with advertised remedies. All these efforts have failed to cure the disease or to affect recognisably its continuance.
- III. There are instances of stocks "passed over" within apiaries, though in close proximity to sick stocks. In this area at least one example is known of a stock prospering in a non-disinfected hive in which a stock had died out of the disease.
- IV. Bees, in a number of instances, were sent by bee-keepers to the editors of the *British Bee Journal*, and were reported to be suffering from Isle of Wight disease. We have been unable to find *Nosema* in the bees of Deeside generally.

III.—INVESTIGATIONS BEARING ON THE RELATION OF *NOSEMA APIS* TO ISLE OF WIGHT DISEASE.

METHODS OF EXAMINATION OF BEES FOR THE PRESENCE OF *NOSEMA APIS*.

As carried out by JOHN INNES, B.Sc., M.B., Captain R.A.M.C.

Each bee was examined in a routine manner, and the following notes were made:—

- (1) Activity, as determined by movements in the postal cage—usually a match-box.
- (2) Power of flight on exposure in open space.
- (3) Dislocation of wings; if present or absent.
- (4) Gait; whether co-ordinated or paretic.
- (5) Response to stimuli, such as handling, etc.

The chyle-stomach and colon of each bee were examined systematically, and the removal of these parts from the body was carried out in the following manner. The head of the bee was firmly crushed between fairly stout dissecting forceps, and while held thus, the last segment of the abdomen was grasped with straight fine-pointed forceps, and gentle traction applied. In this way the colon was carefully pulled out and the chyle-stomach also came away with it. These organs were placed on a slide, separated at their anatomical junction, and teased. Cover-glasses were then applied, and the preparation examined microscopically in the wet, unstained condition. If the spores of *Nosema apis* were present they were readily identified by their shape, size, and refractility. Young, intracellular stages and planonts were also observed in like manner.

Stained preparations from smears of the colon, and of the chyle-stomach, were made from every sample of bees examined, and the stain which was almost exclusively used was the iron-hæmatoxylin of Heideinhain. Although requiring longer time for staining, this was found to give the most reliable and most definite results. The fixative used was a hot alcoholic solution of corrosive sublimate.

Various other stains were tried, such as Giemsa, Romanowsky, Ehrlich's triacid, etc., but the varying results led to their abandonment in favour of the more stable hæmatoxylin preparation.

No stain, however, was found which would show up the spores clearly. Various experiments in this connection were tried, but with no success. Heat had very little effect in helping the stain to penetrate the resistant spore capsule. For its identification in stained preparations we could

rely only upon the surrounding structures being stained and the spore, left untouched, appearing as a clear structure.

The number of bees examined from each stock was, on an average, about eight. Where more than eight bees were available from a stock, then these were also examined.

The amount of infection was reported as slight, heavy, or very heavy, according to the number of spores found.

Where young stages were found, these were recorded as such, but it was rare to find a heavy *young stage* infection without the presence of a fairly heavy infection with spores.

The routine here described was that followed by Dr Innes in the laboratory, in all cases of bees which were sent for *Nosema* diagnosis from the Lewis stocks, from apiaries on Deeside, and several other districts. Examinations of bees for *Nosema* by the other workers was mostly, though not exclusively, confined to the searching for spores. These were obtained by examining bees in the fresh state and by the pulping method described later. Miss Simpson and Dr Rennie also prepared a number of sections of the alimentary canals of *Nosema*-infected bees (apparently normal in behaviour), of Isle of Wight diseased bees, and of healthy bees, some of which are utilised in illustration of the present work.

DIAGNOSIS OF NOSEMA INFECTION IN DEAD BEES.

Graham-Smith, Fantham and Porter, in their report upon *Nosema* as the cause of Isle of Wight disease, express the opinion that dead bees are unsuitable for *Nosema* diagnosis (p. 41). This opinion is reflected in the Board of Agriculture Leaflet, where it is stated that "it is not possible as a rule to express any opinion from an examination of dead bees." Leaving out of the question altogether the problem of Isle of Wight disease, and considering only that of *Nosema* infection, this statement has not been borne out by our experience. Undoubtedly, the most reliable means of diagnosis of *Nosema* infection is the recognition of the spores, and our experience has been that these are recoverable from bees at least as long as seven months after their death. We have in our possession at the present time (January 1916), a number of dead bees which were artificially infected with *Nosema* early in June 1915, in which we are still able to obtain spores, not only from numbers pulped together, but readily from individual bees.

Graham-Smith, Fantham and Porter are of opinion that spores are not often formed in the warmer months of the year. They state: "Undoubtedly

the most certain evidence of the presence of *Nosema apis* is afforded by finding the characteristic spores. Unfortunately these are not always present. . . . Our observations show that during the warmer months the parasites often do not reach the spore stage, but seem to kill the affected bees before this stage is reached" (p. 48 of 1913 report).

Our evidence is unfortunately rather contradictory, as we have been able to find abundance of spores in the months of May, June, July, August and September as readily as in other months. Our illustration of a chyle-stomach in which every visible cell is heavily infected with spores (Pl. I. fig. 2) is from a summer bee. And we are a little doubtful of any heavy mortality resulting entirely from infection in the period of the bees' maximum vigour, at an early stage of the parasites' growth. This is especially so since we have found, throughout the summer, living bees without any obvious signs of disease, which proved to be very heavily infected with spores. Further it is claimed that the chyle-stomach of the bee is, even under normal conditions, constantly shedding and renewing its secreting epithelium.

PULPING EXPERIMENTS.

The routine of examining individual bees for *Nosema* diagnosis, although valuable and most reliable as regards the single bee in question, has several serious disadvantages. The first of these is due to the amount of time required to examine thoroughly even one bee, and the second, which depends upon this fact, is that one is led to base conclusions regarding a whole stock upon the results obtained from the examination of relatively insignificant numbers. A small sample of bees taken at random from a stock does not correspond, say, to a sample of a homogeneous fluid or solid substance.

This difficulty has been largely overcome in our later work by the introduction of the method of pulping collections of bees designed for examination for the presence of *Nosema* spores. Our method is simply to cut off the abdomens of the bees, letting them drop into an ordinary mortar. They are then gently pulped in sufficient water to render the contents fluid. Sometimes we have removed the alimentary canals from behind the honey sac and teased these collectively in a little water in a large watch-glass. Samples of the resulting fluid in either case are taken up with a pipette, and if *Nosema* spores are present in the pulped preparation, they are usually found in the first drop examined. Our first experiment of this nature was as follows.

Four samples of dead bees were taken. Two of these were from collections of bees which had been infected by feeding with *Nosema* spores nearly three months previously, and the remaining two consisted of bees from Isle of Wight diseased stocks.

- (a) Pulp of twenty-six *Nosema*-infected bees yielded, on examination, numerous spores in the first and every succeeding drop of fluid taken from the mass.
- (b) Pulp of a second (separate) lot of *Nosema*-infected bees—about a dozen bees. Result as in case of (a).
- (c) Pulp of bees, "crawlers," from two stocks—different apiaries on Deeside—same stocks as were used for infection in second Shawbost experiment (p. 54)—about twenty bees. Result: No *Nosema* spores observed after repeated examinations.
- (d) Pulp made of about two thousand bees from a stock which had died out from Isle of Wight disease at Banchory. The fluid, after draining off from the solid residue, was tested extensively (twenty separate examinations), and *Nosema* spores were not found.

Other similar tests are given below:

1. Thirty "crawlers" from DES1 stock (p. 57) were picked up on 10th September. They were allowed to die in a small cage, and on 14th were pulped in a little water in the usual way. In this stock there had been found previously an unidentified organism. The first field examined in the first small drop contained numerous spores of this organism. *Nosema* spores were not seen.

2. Sample from Anguston, consisting of nineteen "crawlers," had their abdomens pulped and examined for *Nosema* spores. Negative result.

3. On 11th September 1915, visited Anguston and found nineteen of the stocks had succumbed to Isle of Wight disease since last visit. Took a sample of dead bees from the site of one of the dead stocks.

On 20th September these bees, sixty in number, were pulped, and examined. Nineteen samples were dealt with and no *Nosema* found.

4. On 25th September 1915, seven bees which had died in transit from Glenhouse, Stornoway, were pulped. Negative result as regards *Nosema* spores. These were "crawlers" from the front of the hive, but we have no other record of Isle of Wight disease in connection with this stock. Seven live ones which survived the journey were similarly treated. Result: Negative as regards *Nosema*.

5. Six bees which remained of the lot of Carniolans (No. 7) from Stornoway were pulped on 25th September. No *Nosema* was found.

6. Pulped 27 chyle-stomachs of Welsh driven bees, which were hived to-day in Desswood apiary (29th September 1915), and examined twelve drops for *Nosema*. Result: Negative.

Numerous further tests of this nature have been made, and in every case where *Nosema* was known from previous experience to be present in the collection from which the bees were taken, *Nosema* spores were found.

As early as 1913 we had become doubtful of the conclusion that *Nosema apis* stood in direct causal relation to Isle of Wight disease. From February of that year we regularly examined bees for *Nosema* spores, especially any that crawled or seemed sickly. We found such spores very frequently, particularly in old bees and not necessarily in association with unusual mortality in a stock. We have found bees with a heavy spore infection able to fly perfectly and even to carry loads of pollen and nectar. We have had under observation for three years, 1913-1915, a stock of Ligurians, G9 (Nicolson Apiary), which has stood in proximity to several of the forementioned stocks throughout their periods of crawling. This stock has never displayed any noticeable crawling symptoms. Yet all the time *Nosema* has been present in it, sometimes in enormous quantities (see Pl. I. fig. 4—a microphotograph, taken in the present year, of the colon contents of an apparently healthy bee from this stock).

It is significant that, while G9 contained *Nosema* over a prolonged period, it failed in any marked way to infect the apiary generally. We have not found *Nosema* distributed in the bees of all the stocks, but only in a few stocks at any one time, and where it occurred, Isle of Wight disease was not present.

History of certain Stocks in Lewis—with special reference to the alleged connection between Nosema apis and Isle of Wight Disease.

We visited Bayble on 7th May 1914, and took samples of a stock of bees which had no history of crawling. Spores of *Nosema* were found in several of the bees, the infection being very heavy in a number of cases. On 4th September, when again visited and examined, this stock was in a highly prosperous condition; it filled the brood box of ten combs and crowded also a super of ten shallow frames filled with honey. On 14th August 1915, this stock was still prosperous and had never shown any crawling symptoms. Sample bees from this stock were taken in September, and these were found to be free from spores. Twenty bees were examined: these were lively, vigorous, and flew readily after two days in transit. The chyle-stomachs of

five examined individually were of a healthy appearance; the colons were not markedly congested, and contained watery material consisting almost entirely of indigestible pollen residues. The abdominal parts of the remaining bees were pulped, and drops of the fluid examined as offering the most reliable means of detecting the presence of *Nosema* spores. This stock remained healthy alongside the next-mentioned.

Meanwhile there had been in the same apiary, and in close proximity to this stock, an independent outbreak of Isle of Wight disease. A swarm of black bees arrived from the South of England on Saturday, 11th July 1914, and was duly hived. The bees began to crawl next day, and the symptoms were very marked when we visited the apiary on 13th July. Then, nearly half of the stock was to be seen crawling or dead upon the ground.¹

Bees taken on this occasion were examined in Stornoway by J. A. and J. R. with negative results, and stained preparations were afterwards made without young stages being found. This swarm was headed by an Italian queen, which had been supplied just before the stock was despatched to Lewis. A little patch of brood was hatched, and the yellow bees did not "crawl." The blacks were all dead in autumn and the yellows were not strong enough to winter.

Stock G9: Nicolson Apiary.—This stock has no history of Isle of Wight disease and no abnormal death-rate. A few "crawlers" have occasionally been observed, but nothing to indicate disease, yet this stock is known to have harboured *Nosema* for at least three years. The original queen was a pure Ligurian imported from Bologna (apiary of Enrico Penna), in the autumn of 1911. A black stock on each side of this hive (the stocks already referred to as C13 and D1) died of Isle of Wight disease during that autumn and winter, but the Ligurians showed no signs of trouble. These extinct stocks were replaced in 1912 by two other black stocks (D4 and H12) which died out—D4 certainly, and H12 probably, from Isle of Wight disease before the following winter. But still the Ligurians showed no signs of this disease.

1st September 1912 was very windy, and the hive of G9 was blown right over, the combs being scattered. The bees were gathered up and the queen found uninjured, but this accident handicapped the bees for getting into condition for wintering. The spring of 1913 found them very weak and hardly any of the young bees were able to fly.

Examination for *Nosema* spores gave negative results at this stage. The queen eventually ceased laying, and she was transferred to a queenless black stock. In due course brood appeared, and in this case most of the young bees

¹ Bees from this stock were used in Tolsta experiment (see p. 52).

could fly. A number of those which "crawled" were sent to Dr Annie Porter, together with a description of the symptoms, and she reported as follows:—"Every bee contained spores of *Nosema*." This was in July 1913. The crawling referred to here was not typical of Isle of Wight disease, and lasted for quite a short time. Also the number involved was very limited, and consisted entirely of young Italian bees. At intervals during June, weak and aged bees, both blacks and Italians, had been picked up in front of this hive and examined on the spot, by one of us (J.A.). These were full of spores of *Nosema*, but none were found in the young Italians examined at the same time.

A neighbouring queenless black stock was now united with this Ligurian stock, and soon there was a prosperous colony with plenty of brood, worker and drone. On 12th September 1913, bees taken at random off the frames were examined and no spores of *Nosema* were seen. During the winter of 1913-14 frequent samples were taken, and a large proportion of the bees examined were found heavily infected with spores. Yet those bees were quite active and healthy and could fly perfectly.

On 19th December 1913, the old queen died and stock G9 continued queenless and broodless for over three months. On 28th March 1914, we added a small lot of Italians which had been found starving in another hive. The queen was a Penna Italian imported in 1913. Bees of this stock were found to be heavily infected with *Nosema* on 2nd June.

When we examined the stock on 20th June 1914, it was quite prosperous with a good show of brood. The weather was wet and cold, and diligent search in front of the hive resulted in the finding of eight benumbed bees. Seven of these were full of spores of *Nosema*. Eight bees were also picked off the combs at random, and three of these contained spores of *Nosema*. Again, on 13th July, one bee out of six examined contained an abundance of spores. This stock is still alive at the present time.

NOSEMA INFECTION EXPERIMENTS.

(a) *Infection of a full Stock of Bees with Nosema apis.*

On 11th July 1914, a small lot of Cyprian bees which were known previously to harbour *Nosema* were placed in confinement. On 28th July most had died, and the remainder appeared weak. Four were examined, two live and two dead. All contained *Nosema* spores in quantity. The whole lot of bees, ninety-one in number, were mashed with honey, and fed to a full stock of bees at Achmore, Lewis. These were ordinary black bees imported

as a swarm from the South of England in June 1914. They had been examined on 30th June, and again on 13th July, and found negative as regards *Nosema* spores and young stages. There were no other hive bees within a radius of $5\frac{1}{4}$ miles.

On 19th of August, the stock was visited and examined. It covered nine combs, and clustered on the dummy. Not a "crawler" nor dead bee was to be seen in front of the hive, although the weather was very favourable for this. Ten bees, taken at random, were examined for *Nosema*, and spores were found in four, two of these showing a very heavy infection.

On 7th September, a second sample consisting of twelve bees was examined, again taken at random from within the hive, and five were found infected, three of them heavily. The day was fine, and there were no indications of Isle of Wight disease.

On 10th October, the bees were again visited and samples taken, from inside the hive as formerly. It was a fine October day, and the behaviour of the bees was normal.

On 13th October nine of the sample bees were examined. They were active, flying readily, and their intestines were normal in appearance. No spores of *Nosema* were found. On the 14th, eight more bees were examined, again with negative results. On the 22nd, three bees of another sample were taken. These arrived alive and active. On examination they were found to have the intestine normal, and to contain no spores of *Nosema*.

From the foregoing it appears certain that this stock of bees was definitely infected with *Nosema*, but this infection failed to produce Isle of Wight disease.

The following are illustrations of numerous artificial infections with *Nosema* carried out on small lots of bees confined in cages.

(b) *Laboratory Experiments.*

I. *On 14th June 1915.*—*Nosema* spores derived from bees of Stornoway, stock No. 7 (Carniolans), were used to infect some bees taken from an observation hive at Marischal College, Aberdeen.

On the 17th, *Nosema* spores were found in plenty in five bees taken at random from the infected lot. These spores were in alimentary canal; no intra-cellular forms were seen. Our view is that those seen were the spores ingested.

19th June 1915.—Numerous spores were found in the colon of one dying bee, and a few in the chyle-stomach. Young stages were observed in cells of chyle-stomach (J. A.).

21st June.—A dead bee from infected lot was examined; spores were found in colon, but not in chyle-stomach. Many cells of the chyle-stomach showed intra-cellular stages (J. A. and J. R.).

1st July 1915.—On 30th June, two bees were found dead. When examined on this date a few spores were found in both, and a great many young stages were seen, especially in one bee. A stained smear showed many meronts within the cells, there being frequently four parasites in one cell (J. R. and J. A.).

This infection experiment, through pressure of other duties, was allowed to come to an end about this time. But the dead bees were preserved, and in September they were found to contain *Nosema* spores in great abundance. At this latter date, however, several attempts to produce infection by means of these spores failed.

II. *20th September 1915.*—On this date thirty bees from *Craibstone No. 41* (a stock originally Italian, but now consisting entirely of black bees) were confined in a small cage, and fed on spore-infected material mixed with honey. The spore material was obtained from the 14th June experiment. Five bees were killed and their chyle-stomachs pulped and examined, before infection, for *Nosema* spores. Result—negative (J. A. and J. R.).

On 21st, the bees were again fed with the spore-laden material. Up to 25th the behaviour of the bees was normal; they fed freely, and were quite active. There had been no mortality except in the case of three, which died on the 21st. In one of these ingested spores were found.

On 26th, one bee died and one was killed. No spores were found in either of these.

On 28th September, two died and two were in a weakly state. No spores were found in any of these.

On 29th, one died and no spores were found. The bees were fed on Scholtz candy.

The intestine in the last examined bee was whitish, and not unlike the appearance of a *Nosema* infection, but, as stated, spores were not observed.

On 7th October, fourteen bees which had died were pulped. These were black bees: no *Nosema* spores were found.

It is clear from the foregoing that the spores used in this experiment failed to infect.

III. *On 30th September*, a small lot of Welsh bees were caged and fed on *Nosema*-infected material of 15th June. Spores were present in the material fed. A similar number of bees were caged as a control, and fed on clean candy. Feeding with the infected material, in the case of the first lot, was continued on the 1st and 2nd October, after which clean candy was given.

On 7th October, it was found that sixteen bees of the infected lot had died. These were pulped (abdomens) and examined for *Nosema* spores. Free spores in moderate numbers were found; two or three in every field examined of the first drop taken. One live bee examined gave a negative result. In the control, two bees had died. These were pulped (abdomens only), but no spores were found.

16th October 1915.—Five bees dead. These were pulped on 18th, and *Nosema* spores were found. Other three were examined, and spores found in all. Other six were pulped, and spores were again found. All the control bees had died at this date.

From these results it seems probable that no infection had taken place.

IV. 4th January 1916.—On this date all the Desswood stocks were in flight. The opportunity was taken to procure a number for experiment, and samples were captured on the alighting board of the "Kelly" hive. These were fed with candy, which on the same day was well smeared with pulped matter from bees of the June experiments. This matter was found on preliminary tests to contain *Nosema* spores in plenty.

The bees were kept in a small wooden cage with glass cover. By the 6th several had died. Nine were removed at this date, but of these, two were dead shortly after caging, having probably been hurt.

6th January 1916.—Two were allowed out of the cage, and these flew quite strongly, and one of them defecated.

8th January 1916.—Other six bees were found dead. One released proved unable to fly, although it was quite active. When lifted it used its sting. This bee had chyle-stomach, pale in colour, and colon dilated with a pasty mass of pollen. Search in the dead bee and in the surviving one failed to find any trace of *Nosema* spores. Another live bee very active was taken, killed and examined. This bee had a pale chyle-stomach and inflated watery colon. Numerous free spores were found in the latter, but a very thorough search failed to discover any trace of infection in the chyle-stomach.

12th January 1916.—On the 12th all the bees had died. These (thirteen) were pulped, and examination showed free *Nosema* spores in moderate numbers.

V. 24th June.—Infected two boxes of "Kelly" bees with *Nosema* material from No. 7 Stornoway hive.

1st July.—Examined two bees which had died. Planonts seen in both bees and intra-cellular forms in stained film. No free spores seen.

8th July.—On this date the chyle-stomach of a bee of this experiment, taken alive, was found on examination to contain enormous numbers of spores and planonts. Spore clusters common.

VI. *20th June*.—Fresh spores of *Nosema* were, on this date, mixed with honey and fed to a number of bees in a small cage. On the 26th several of the bees were killed, and an examination was made of the alimentary canal. Planonts were seen in very large numbers. A further examination on the 30th revealed the presence of both planonts and meronts. Spores were not seen.

In connection with the foregoing experiments control lots of bees were in most cases kept. Further we have had a very considerable experience with caged bees under the same conditions as those of the experiments. We have not been able to detect significant differences in mortality amongst such caged bees. No conclusions are therefore drawn with reference to the cause of death. Our experience is that captivity in small cages without a queen is eventually fatal to the healthiest of bees.

With regard to the behaviour of the bees while under experiment, it was not possible to decide whether Isle of Wight was present. The condition of the intestine which we have learned to associate with Isle of Wight disease, and which we find has been described by Imms (*Jour. B. of A.*, xiv. pp. 133-4), has never been observed in those caged bees.

The experiments numbered II.-IV. were made in September and January with spores obtained from bees which died in June. These dead bees were kept in a laboratory cupboard under ordinary conditions, and the results would suggest that in some instances at least the vitality of the spore is brief.

CONCLUSIONS REGARDING RELATION BETWEEN NOSEMA APIS AND ISLE OF WIGHT DISEASE.

Our main conclusion regarding *Nosema apis* is that so far we have been unable to recognise any causal relation between the presence of this parasite and the disease. We have found it to be present over prolonged periods in healthy stocks, while we were unable to find it in other stocks in the apiary, nor did Isle of Wight disease spread under these conditions although various races of bees were present. Deliberate infection of a stock with *Nosema* did not produce the disease. It is well established also that the disease occurs where the parasite cannot be found. We have numerous instances of this on Deeside.

From known facts regarding *Nosema* in other countries, *e.g.* in Germany and in Victoria, Australia, it appears that *Nosema apis* is just as common in healthy bees as in diseased. They are practically all "parasite carriers."

We recognise that *Nosema* may be a contributing weakening factor favouring in certain cases the development of this disease. But we have not found that it is an essential factor.

IV.—OBSERVATIONS AND EXPERIMENTS BEARING ON THE INFECTIVITY OF ISLE OF WIGHT DISEASE. AS DISTINGUISHED FROM MICROSPORIDIOSIS.

(a) ATTEMPTS TO PRODUCE ISLE OF WIGHT DISEASE BY ARTIFICIAL INFECTION.

No. 1. Tolsta Experiment. July 1914.

The stock utilised in this experiment was a small swarm of unknown race, originally imported from the South of England. It was transferred for the purpose of this experiment from the Nicolson apiary to Tolsta which is six miles from the nearest hive of bees. A control examination gave a negative result as regards *Nosema*.

On 18th July 1914, three large lots of "crawling" bees, numbering hundreds, were taken from the stock at Bayble suffering from Isle of Wight disease (see p. 46). These were beaten into a compost with honey and spread over the tops of the combs of the Tolsta bees. When the hive was visited on the 27th July everything had been cleaned up by the bees. There was no trace of the honey nor of the mashed-up bodies. The bees were multiplying and required extra combs.

On 19th August, they covered fully eleven combs and had gathered considerable stores of honey. Not a "crawler" was visible, nor even a single dead bee to be seen in front of the hive. Samples at this date were examined by Innes who reported:—"Bees alive, active. No *Nosema* spores found."

On 12th September, the bees were found to fully occupy twelve combs, and not a dead bee was to be seen. There were no indications of any kind suggestive of Isle of Wight disease.

On 17th October, one fully sealed outside comb of honey was removed leaving eleven combs loaded with honey. The bees were in excellent order.

On 7th November, the Tolsta bees were again visited. It was a fine mild day and the bees were flying freely. Several drones were observed in flight, but these were being attacked by the workers. The presence of drones so late in the season probably indicated that the queen was

failing. There was no crawling or other symptoms of Isle of Wight disease.

On 2nd January 1915, the stock was visited and found normal.

On 13th March, the stock was found lively and vigorous.

On 28th April, the bees were found numerous and lively; there was a patch of drone brood in worker cells suggesting the presence of a drone-laying queen. Probably the aged queen had died during the winter leaving eggs and young brood, from which the bees were able to raise a young queen. This queen could not be mated, however, at this season. This drone breeder was actually found on 3rd May and removed. After some difficulty a fertile queen was again established in the hive and the stock continued its prosperous career, giving, in July 1915, a natural swarm, which was lost.

On 8th August, the swarmed stock was found queenless and broodless and much reduced in numbers, but lively and vigorous, and with much stored honey. The stock was re-queened on 10th August with a young American Golden queen bred in Stornoway.

This stock, in spite of a heavy contamination with fresh Isle of Wight material, remains free from the disease to this date, January 1916.

No. 2. Shawbost. First Experiment. October 1914.

The bees which were the subject of this experiment were Carniolans, and the stock had swarmed in July 1914. It was headed, therefore, by a young queen, and occupied seven combs.

On 1st October, two ordinary match boxes of "crawlers" from an infected stock (60 DD, see p. 56) were sent to Lewis from Aberdeen. The crawlers were taken from a lot which came out in great numbers on a sunny afternoon when the "Isle of Wight" signs were most typical. They were picked up after having been out over night, and despatched by post alive, with food. These crawlers were mashed up on arrival, mixed with honey, and fed to the stock at Shawbost, on 3rd October.

On the 10th October, the bees were reported to be in fine order with brood on two combs. All infected material had been cleaned up by the bees, and not a dead bee was to be seen.

On 24th October, the local observer reported: "No sign of any disease."

On 20th February 1915, the stock on examination was found to be in good condition, and no dead were visible.

On 27th April, the stock was reported to be in fine condition, the bees were lively and "vindictive," and there was a good deal of brood.

At the beginning of June 1915, the stock was reported to be rather

weak in numbers but it built up rapidly, and on 12th August the report was as follows: "The seven original combs are now quite inadequate, and the bees have passed behind a double dummy and have built a considerable mass of new comb which is filled with honey. This is a highly prosperous stock."

On 4th September, the stock was still in excellent condition. At this date eleven months had elapsed since the Isle of Wight disease material was served up to the bees, and throughout the whole of that period the bees had been perfectly healthy, and no infection had resulted from the experiment.

No. 3. Shawbost. Second Experiment. September 1915.

On 24th August 1915, the colons and chyle-stomachs of twenty-five crawling bees were taken from front of hives No. 10 of K and the worst stock of H, Finnercy. Both stocks were very bad with Isle of Wight disease. The day was warm and sunny, and there were many hundreds of "crawlers" about in both cases. The intestines of all the bees (twenty-five in number) were drawn out, and in every case the colon was congested with pollen in a pasty condition. Most of the chyle-stomachs were purplish. These were laid out in a row and allowed to dry—and on the following day heaped together.

The above-mentioned bee-material was mixed in a little water and twelve samples examined. It consisted chiefly of pollen grains, a good many of which were digested. A few epithelial cells of the chyle-stomach in various stages of disintegration, some still showing the zymogen granules present, were seen, also some which seemed intact. None of these showed any sign of *Nosema* infection, and no free spores were observed.

Bacilli, long and short, and also cocci were seen in the preparations. This material was mixed with candy and sent to Lewis the same day, viz. 30th August. The local observer (Ewen) reported that on 4th September he fed this to Shawbost bees, and describes the bees at this date thus:—"I placed the compost on the top of the frames. The day was fine and the bees were very active. The stock in the beginning of June was very weak in numbers, but has done very well during the summer." This stock is still under observation, January 1916, and apparently healthy.

No. 4. Back Bees, Stornoway. June 1915.

In this experiment old combs and hives were used. The bees originally occupied only one of two hives, the other being left open in the hope that

any swarm might hive itself. The nearest bees to Back are at Tong, about 2 miles away, and the Tong bees have always been quite healthy.

At June 1915, the Back bees were so prosperous that they were supered. On 28th June, the super was removed and the tops of the frames smeared with a paste made by mashing diseased bees with honey. These bees were crawlers picked off the ground in front of an infected Deeside stock. The bees were sent alive from Aberdeen and pulped in Stornoway.

On 8th August the stock was visited, and it was found that the bees and the two hives had been shifted from the original site to a less suitable one in a very unskilful manner, by one who was not a bee-keeper. The bees had previously swarmed, and the swarm had occupied the vacant hive as had been expected. The combs and quilts had been disarranged, so that the swarm got a very poor chance. The jar used in infection was still on the parent stock and some comb had been built in it, but there was no trace of the Isle of Wight material.

On 14th August, both lots of bees had to be hurriedly shifted to still another site at a distance of about 400 yards, and during the process a considerable number of bees was lost. There was no brood in either hive; presumably both were headed by virgin queens. This absence of fertile queens, the two disastrous removals, and the swarming constituted a combination of factors which could not fail to weaken and discourage the bees.

On 19th June, the bees had been examined microscopically; no spores of *Nosema* were found.

On 11th September, bees from Back arrived in Aberdeen. They were active, and some of them survived until 16th. On this date live bees were examined individually for *Nosema* with negative results. The dead bees were pulped, and samples examined were also found free from *Nosema* spores.

On 20th October, bees were received which had been dispatched on the 12th, with the following report:—"Symptoms same as No. E14 in Nicolson Apiary. The stock is almost exterminated. The bees sent represent 'crawlers' and remnants in the hive."

Of these fifteen dead bees were pulped, and drops examined for *Nosema* with negative results.

On 28th October, Back was visited and both stocks were found almost extinct. A small knot of bees remained in each hive on the combs. The queen of one was warmed into activity, and lived for a few hours. There was plenty of food. The local observer (Morrison) reported that both lots had displayed the typical "crawling" symptoms.

It is worth noting that while this experiment has culminated after four months in the bees apparently contracting Isle of Wight disease, which did

not occur in other two similar experiments, it is not clear that this was due to the artificial infection. There were specially adverse circumstances in the present case. In the other two instances of attempted infection, the bees were maintained in good general external conditions. It should be noted further that *Nosema* was not found at any time during the experiment.

The foregoing experiments in artificial infection were made in Lewis upon isolated stocks. The following were carried out in Aberdeen.

No. 5. 60 DD. May 1915.

This stock, which stood in an apiary in a district suffering badly from Isle of Wight disease, was found, on 24th September 1914, to have large numbers of bees on the alighting board, and groups of "crawlers" on the grass around. The weather conditions were fine and sunny. The same thing was observed on the following day, and the hive and bees were removed to an observation area in Aberdeen. For a few days after this the stock seemed to be doing well enough and a good deal of pollen was being taken in. On fine sunny days, however, "crawling" became very evident, and on some occasions it was very extensive. As recorded elsewhere a sample of the "crawling" bees was utilised in an infection experiment (p. 53).

On 7th October, which was a fairly good day with a little sunshine, "crawling" was again in evidence, but pollen was still being gathered. About this time it was reported that three other stocks from the same apiary were suffering from the disease. All these eventually perished (p. 34).

Up to 17th October, bees "crawled" every fine day, but the last of them perished in the early winter, leaving abundance of stores in the hive.

On 19th May 1915, a fresh stock arrived from the South of England, Italian hybrids, fully covering ten combs crowded with brood. The queen had apparently been recently lost, but there were numerous queen cells on the combs. The new stock was put in the hive in which the above-mentioned stock, 60 DD, had been housed. There was no disinfection of any kind, but the dead bees and the old combs were removed. The honey was drained from some of those old combs and fed to the new stock.

On 3rd June, a swarm came off and was duly hived in a perfectly new hive on frames with full sheets of foundation. This swarm was also fed on honey drained from the old combs left by stock 60 DD.

On the afternoon of the following day a second swarm came off and was hived in a straw skep. This second swarm received none of the honey from the old combs, but was fed on a little honey bought in the open market.

Eggs were found in the parent stock (DEP) on 14th June, and the queen of the first swarm (DES 1) began to lay on the 15th.

The queen in the second swarm was long in being mated, and was balled by the bees on 28th June. A black virgin queen was introduced on the 30th June, and she began to lay in due course. The other two queens were as yellow as pure Italians, and the young bees were also light coloured.

The population of the parent stock was much depleted by the double swarming, and further, on 23rd June, foul brood was found to be present. This was a variety which attacks the larvæ before sealing, and attempts were made to treat the infected cells with dilute formalin. This treatment was successful so far in that the disease appeared to be checked, but unfortunately the queen was lost either during the manipulation, or poisoned by the formalin vapour. Queen cells were made from the brood in the hive and a black queen was produced, which ultimately began to lay. The stock was now very weak in numbers but was active in raising brood, in defending the hive against robbers, and in carrying pollen.

Up to 4th September it could not be said that there were any symptoms in the apiary of Isle of Wight disease. From the nature of the experiment here being recorded, a specially close watch was kept for indications of its appearing. Only at one period were suspicions aroused. The month of July was very wet and must have been very trying to the bees. On the 25th, a few "crawling" bees were seen in front of the parent stock and also the first swarm, as well as in front of another stock of black bees not related to the bees of the present experiment. And again, on the 31st, it is recorded that there were a few "crawlers," most in front of the first swarm (DES 1). August was also a wet month, but nothing of a suspicious nature was noticed. In spite of the very adverse circumstances above narrated, all three stocks remained perfectly healthy, and up to this date (20th September) the parent stock, which is housed in the presumably infected hive, was fed with honey from this hive, and was later given some of the original combs, is healthy and, as far as the eye can judge, growing in prosperity.

On 30th September, a lot of driven bees from which the queen had been removed was imported from North Wales and added to the little stock (DEP). The bees were fed on sugar syrup boiled with vinegar and were packed down for winter.

On 14th October, the first fine day since the addition of the Welsh bees, large numbers of bees were seen out upon the alighting board. This was probably related to robbing, which was being actively attempted at several hives in the apiary.

On the 28th December, a mild day, with a few bees offering to fly, the hive

was opened, and the quilts were found in a sodden condition owing to a defective roof. The bees, however, were in good condition with plenty of stores.

It has already been mentioned that "crawlers" were observed on two occasions in July in front of the hive occupied by the first swarm. From that date until the beginning of September there were no definite symptoms. August, like July, was mostly wet, and the Italians with their usual improvidence had converted practically the whole of their stores into brood. From 4th September onward this stock, however, now showed undoubted signs of the disease. The first half of this month was uniformly fine, with a great deal of sunshine, and on every occasion the alighting board and the grass in front was covered with numbers of "crawling" bees. Some of these were fairly active and could perform short flights but not sustained ones. Those which left the board were, as far as we could see, unable to return to the hive and crawled about, sometimes as far as twelve yards at least from the hive, ultimately dying on the ground. Those near the hive tended to form small clusters as the day advanced. The alighting board was always cleared in the course of the afternoon. All this is of course typical of Isle of Wight disease. Further, the internal appearance was that characteristic of this disease, although all our microscopical examinations failed to reveal the presence of *Nosema*. The appearance within the hive during these days was practically normal, pollen was being carried in, and there were no indications that the disease was making rapid progress. From 4th September sugar syrup was supplied to all the stocks, and this was freely appropriated by the bees and stored.

The question naturally arises here, Had this outbreak any connection with the proximity of the infected hive or the fact that this swarm came from it? In view of the prosperity of the parent stock which had continuously occupied the hive for four months, and the length of time, viz. three months, since the swarm constituting this stock left the hive, it does not seem likely. But we must admit the possibility of infection having been transferred and remaining latent until conditions favourable for its development within the bees had arisen.

No further "crawling" symptoms have been observed since the beginning of October. On 20th October, a large lot of Welsh bees (driven, queenless) was added to this stock. It is probable that these killed the queen, for on 1st November several dead yellow queens were found on the floor of the hive, and there were queen cells on one of the combs. No free virgin queen was observed, and another queen was caged over the stock. This queen was found on the alighting board a few days later. This stock, therefore, has a virgin queen (grand-daughter of the original English queen).

The second swarm, standing alongside the first, has never displayed any symptoms of the disease.

(b) NATURAL OCCUPATION OF ISLE OF WIGHT AFFECTED SITE.

The Hive and its Contents as Possible Sources of Infection.

The following case is related to the foregoing experiment in respect that it is a naturally occurring instance in which the same factors, viz. hive and combs of an extinct Isle of Wight diseased stock, are involved.

Bees which occupied a frame hive began to exhibit "crawling" and other symptoms of Isle of Wight disease in the autumn of 1913, and the last survivors perished in the spring of 1914. The hive was not disinfected nor the honey and dead bees removed, but the door was closed. In June 1914, a vagrant swarm alighted beside the hive and was allowed to enter. No particular attention was paid to them, for it was not expected they would survive. But it was observed that the new-comers were very diligent in removing the dead bees and getting the hive in order. The bees wintered in good order, and in May 1915 they swarmed. This top swarm was unfortunately lost, though followed for some distance, but a second swarm on 7th June was duly hived in a skep and placed alongside the parent stock. Both stocks were visited and examined for eggs on 12th, 15th, and 21st June, but none was observed. Up till this time both stocks appeared perfectly healthy and were working normally, except that the parent stock had only a small number of bees, and drones were unusually numerous. Thus it will be seen that in the case of the parent stock a full year had elapsed since the bees took possession of a hive and combs with a recent history of Isle of Wight disease.

On the 26th of June "crawling" symptoms were developed in the bees of the swarm, and these were very marked when the stocks were visited on 28th June. The garden in front of the skep for a considerable distance was covered with crawling bees, with characteristic grouping very noticeable. The internal appearance of the crawling bees was quite characteristic of Isle of Wight disease, and we satisfied ourselves that this disease was present. It should be added here that both in 1914 and 1915 the whole surrounding district had been swept by the disease, and there were very few stocks surviving in it. Examination of the combs showed that both queens were laying, and there was a good deal of sealed brood in the skep.

The crawling symptoms above noted became less noticeable from day to day. There were, however, fluctuations, till on 23rd August both stock and

swarm appeared to be free of the disease and were working normally. The skep being overcrowded had been provided with an "eke." On the 11th September, both stocks were found apparently in perfect health. The bees in the skep had extended their combs down into the eke.

All this time there had been no symptoms of Isle of Wight disease in the parent stock.

On 6th October, the parent stock in the bar-frame hive was found rather weak in numbers, and the bees in the skep were driven and united with it. The queen of the skep was removed and introduced to a stock in Desswood apiary. The combs of the skep were cut out and piled over the frames in the wooden hive.

On 29th December, the bottom of the hive was found to be thickly covered with dead bees, but there were bees still alive amongst the piled combs just under the quilt.

It may be noted that to date bees have been living in this hive on the old "Isle of Wight" combs for over 18 months without any symptoms of the disease appearing, and the conclusion is legitimate that the appearance of Isle of Wight disease in the swarm is to be traced to a separate source.

There has also come under our observation the case of a bee-keeper near Perth, who had lost several stocks from the disease, but had several remaining which were apparently in excellent condition, although he had fed them on the stores left by the diseased stocks. One in particular, through an accident, had been soaked with water during last winter (1914-15), and by spring the bees had been reduced to a handful. We examined this stock in October 1915, and found it in fine condition. The bees were black, but the owner declared they were Italians because they had had an Italian queen three years previously. Some of the surviving stocks displayed yellow bands, and the bee-keeper attributed their resistance to the admixture of Italian blood.

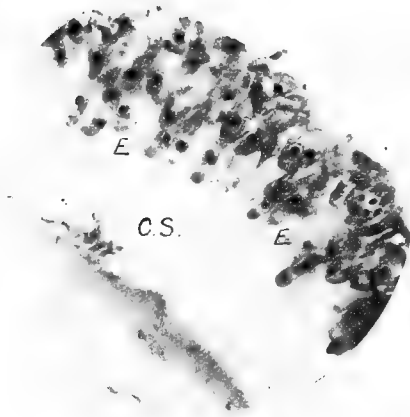
Of direct interest in connection with our failure to produce Isle of Wight disease by means of presumably infected hives and combs is the experience of Beuhne, of Victoria, as given in *The Australian Bee-keeper*, 15th August 1915. He says: "When a very heavy mortality occurred amongst the bees in the Grampians in 1909-10, and microscopic examination showed the parasite in the bees of the apiaries concerned, the question arose whether bee-keepers should follow the directions of the British Board of Agriculture, and destroy all the remaining bees as well as all the combs, or give the survivors a chance, and take a risk with the combs of the defunct colonies.

"My personal experience with this kind of mortality was in 1900-01, when

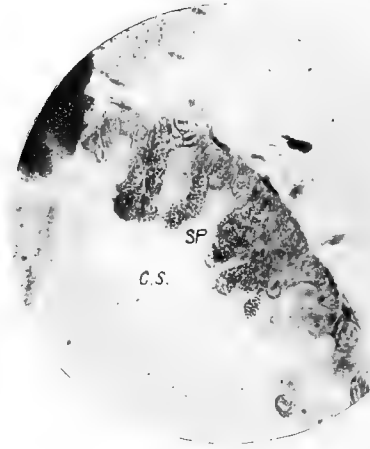


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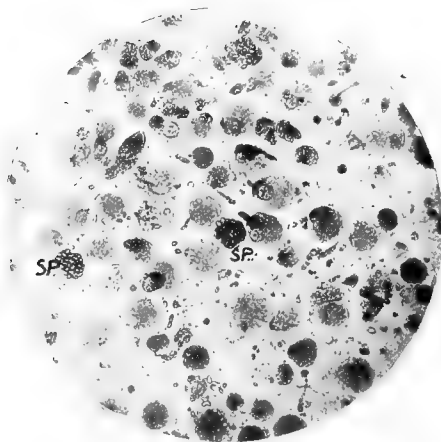
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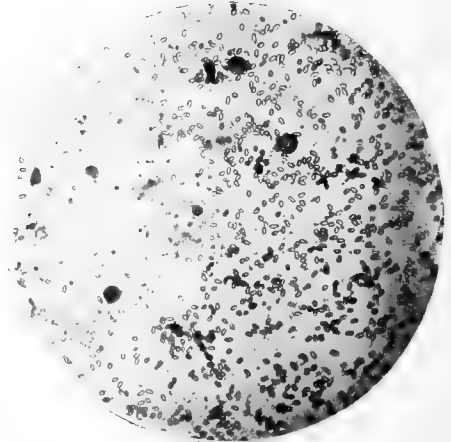
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nothing was known of *Nosema*, and therefore I was unaware of any risk, and used the combs from which the bees had disappeared, when restocking the apiary with purchased bees, and 'nothing happened.'"

Beuhne is of opinion that "under ordinary conditions, that is, conditions favourable to bees, the parasite is merely a casual inhabitant of the intestine of the bee."

GENERAL CONCLUSION REGARDING INFECTIVITY.

The general conclusion to which the foregoing facts point is that Isle of Wight disease, although probably an infectious disease, is one which requires the coincidence of other and presently unknown external factors (besides a specific organism) before the disease develops. The disease is not necessarily conveyed by mere contact with contaminated hives or combs, or by feeding upon contaminated stores.

EXPLANATION OF PLATE.

- FIG. 1. Section of the chyle-stomach of a bee displaying symptoms of Isle of Wight disease. Spores of *Nosema* are not present, and no young forms were found in this outbreak. $\times 180$ (Iron-hæmatoxylin).
- FIG. 2. Section of the chyle-stomach of a bee infected with *Nosema*, but not suffering from Isle of Wight disease. The stained spore contents came out as black dots in the photograph. $\times 180$ (Iron-hæmatoxylin).
- FIG. 3. Portion of the teased chyle-stomach of a bee, showing detached cells, some containing spores of *Nosema*. Fresh preparation. $\times 180$.
- FIG. 4. Fresh unstained preparation, showing *Nosema* spores lying free in the gut. $\times 180$.
- FIG. 5. This shows the behaviour of a stock (E14) suffering from Isle of Wight disease. Note the bees, incapable of flight, clustering on the hive front. Large numbers on the grass cannot be seen, but some can be observed climbing up the leg of the hive.

- c.s.* Chyle-stomach of bee.
e. Epithelium lining chyle-stomach.
sp. Spores and epithelium of chyle-stomach.

(Issued separately, 14th September 1916.)

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